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## Platform Z: Mechanosensitive & TRP Channels

### 1179-Plat

#### Outer Pore Domain of TRPV1 Ion Channel is Required for Temperature-Independent Step During Temperature-Activation

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TRPV1 is the founding and best-studied member of the family of temperature-activated transient receptor potential ion channels (thermoTRPs). Voltage, chemicals, and heat allosterically gate TRPV1. Molecular determinants for TRPV1 activation by capsaicin, allicin, acid, ammonia, and voltage have been identified. However, many years after the discovery of TRPV1, the structures and mechanisms mediating temperature-sensitivity remain unclear. Recent studies of the related channel TRPV3 identified residues within the pore region required for heat activation. Here we describe both random and targeted mutagenesis screens of TRPV1 to identify single point-mutations that specifically affect temperature-activation. The mutations found are all located in the outer pore region, in close proximity to but distinct from residues previously implicated in acid-activation. Electrophysiological analysis shows that mutations affect a temperature-independent step that is part of the temperature-gating pathway. These results suggest that the outer pore plays a general role in heat-sensitivity of thermoTRPs.

### 1180-Plat

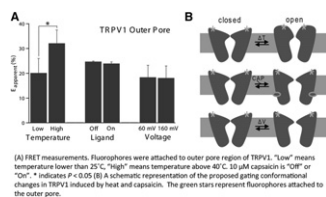
#### Temperature-Driven Activation of Thermotrp: A Distinct Pathway Involved

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A group of thermosensitive transient receptor potential (ThermoTRP) channels, with high temperature sensitivity of channel gating, are the cellular temperature sensors. ThermoTRPs are polymodal receptors. Besides temperature they are gated by voltage, ligand, extracellular pH and other stimuli. How temperature changes drive activation conformational rearrangement remains unknown. Here we combine functional, mutational, and site-directed fluorescence studies to demonstrate that temperature-dependent activation uses a pathway distinct from those for ligand- and voltage-dependent activation.

We observed that neither strong depolarization nor application of capsaicin could significantly alter thermodynamics of temperature-driven TRPV1 activation. In addition, voltage and ligand exhibited additive gating effects over temperature gating. Indeed, a TRPV1 mutant in which part of the outer pore region was replaced by an artificial sequence showed virtually no temperature sensitivity but maintained near normal capsaicin sensitivity. Furthermore, site-directed FRET measurements showed that conformational changes in outer pore can only be induced by heating, but not by voltage or ligand. Together these observations suggest that a distinct pathway for temperature to gate TRPV1 involves the outer pore region.



### 1181-Plat

#### Role Of Pip2 On Ca<sup>2+</sup>-Dependent Desensitization of Trpv2

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TRPV2 is a member of the transient receptor potential superfamily of ion channels involved in chemical and thermal pain transduction. Unlike the related TRPV1 channel, TRPV2 does not appear to bind either calmodulin or ATP in its N-terminal ankyrin repeat domain. In addition, it does not contain a calmodulin-binding site in the distal C-terminal region, as has been proposed for TRPV1. Importantly, though, we have found that TRPV2 undergoes Ca<sup>2+</sup>-dependent desensitization similar to TRPV1, suggesting that the mechanism of desensitization may be conserved in the two channels. To elucidate the

molecular mechanism underlying Ca<sup>2+</sup>-dependent desensitization in TRPV2 we used whole-cell recordings of F-11 cells transiently transfected with TRPV2. We found that prolonged applications of the TRPV2 agonist 2-APB led to nearly complete desensitization of the channel in the presence of extracellular Ca<sup>2+</sup>. In contrast, no desensitization was observed in the absence of Ca<sup>2+</sup>. TRPV2 desensitization was not altered in whole-cell recordings in the presence of calmodulin inhibitors or upon co-expression of mutant calmodulin, suggesting that CaM does not play a major role in Ca<sup>2+</sup>-dependent desensitization of TRPV2. Interestingly, simultaneous confocal imaging and electrophysiological recording of whole cells expressing TRPV2 and a fluorescent PI(4,5)P<sub>2</sub> binding probe showed a high degree of temporal correlation between the Ca<sup>2+</sup> induced desensitization and depletion of PI(4,5)P<sub>2</sub>. Thus, Ca<sup>2+</sup> influx through TRPV2 is sufficient to trigger a dramatic decrease in PI(4,5)P<sub>2</sub> levels, presumably by activating PLC. We propose that the decrease in PI(4,5)P<sub>2</sub> levels upon channel activation underlies at least a major component of Ca<sup>2+</sup>-dependent desensitization of TRPV2.

### 1182-Plat

#### TRPM8 Cation Channel. Effects of Voltage, Cold and Menthol on Single-Channel Gating

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Single-channel patch-clamp recording allows mechanistic insights into fundamental ion channel properties. Thus, a single kinetic model can formally describe the complex interactions during poly-modal activation of the channels. For TRPM8, activation depends on temperature, voltage and chemical signaling [1, 2], and such modeling provides the most comprehensive approach to the study of the channel. In this study, we examined the influence of voltage, cold and menthol on TRPM8 gating using patch-clamp recording techniques. In HEK293 cells stably expressing TRPM8, single-channel currents were measured (filtered at 2 kHz and sampled at 10 kHz) in cell-attached patches at different voltages (−100 to 140 mV), temperatures (20 or 30°C) and menthol concentrations (10 or 100 μM) (n = 7-11). As has been reported for whole-cell TRPM8 currents [3], shifts in the voltage-dependent single-channel open probability curve toward less positive potentials were induced by cold or menthol. Thus, the potential for half-maximal activation was reduced from 162.4 to 116.1 mV during cooling from 30 to 20°C, with a further shift to 52.8 mV with 100 μM menthol. To investigate the relationship between these modulators, we used different techniques - HJCFIT [4], QuB [5] and 2D fitting [6] - to develop a single-channel kinetic model aiming to identify the most likely potential-, menthol- and cold-regulated transitions. A model with 5 closed and 2 open states showing correlation between brief openings and long closings and between brief closings and long openings, was able to describe our macroscopic and single-channel data. Interestingly, temperature and menthol mimicked voltage-dependent activation of the channel at the model level by increasing the probability of transitions from long closed states to brief ones. This is the first complete kinetic model based on single-channel data for any of the TRP channels.

### 1183-Plat

#### Phosphoinositide Regulation of TRPM8 Channels in Planar Lipid Bilayers

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The cold and menthol receptor TRPM8 is regulated by membrane phosphoinositides. To study the effects of lipids directly on the channel, we have reconstituted the purified TRPM8 in planar lipid bilayers. This system allows full control of the lipid composition in our experiments. The reconstituted channel was activated by menthol or cold, and its activity depended on the presence of specific phosphoinositides. In the presence of menthol, TRPM8 exhibited the highest probability of opening in the presence of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P<sub>2</sub>] with Po ~ 0.89 at +100 mV and Po ~ 0.4 at −100 mV. Less channel activity was induced by phosphatidylinositol 3,4-bisphosphate [PI(3,4)P<sub>2</sub>] with Po ~ 0.53 at +100 mV and Po ~ 0.2 at −100 mV. Phosphatidylinositol 3,4,5-bisphosphate [PI(3,4,5)P<sub>2</sub>] resulted in irregular TRPM8 channel currents and lower open probability with Po ~ 0.21 at +100 mV and Po ~ 0.087 at −100 mV. Among the tested lipids the lowest TRPM8 channel activity was induced by phosphatidylinositol 4-phosphate [PI(4)P] with Po ~ 0.12 at +100 mV and Po ~ 0.019 at −100 mV. The lipid specificity profile in lipid bilayers is very similar to that observed in excised patches. We have also studied the activation of TRPM8 channels in lipid bilayer with cold. Cooling the system with reconstituted TRPM8 channels also required the presence of PI(4,5)P<sub>2</sub>. The main shift in the channel behavior was observed in the temperature range from 21°C to 18°C where the channel showed drastic changes in the open probability from 0.05 to 0.85 at +100 mV.